TRANSLATION NO. 2396

DATE: 1967

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THE EFFECT OF PLAGUE MICROBE TOXIN ON THE ACTIVITY OF PYRUVATE-OXYDASE AND LACTATEDEHYDROGENASE IN PLAGUE-SUSCEPTIBLE ANIMALS

[Following is the translation of an article by Z. I. Vasilyeva, Irkutsk Scientific-Research Anti-plague Institute for Siberia and The Far East, published in the Russian-language periodical Voprosy Meditsinskoy Khimii (Problems of Medical Chemistry) 13(2), 1967, pages 124--30. It was submitted on 21 Jul 1965.]

In our earlier investigations we established certain peculiarities in the oxydation of pyruvate in the organism of white mice during plague intoxication [1, 2]. It has been demonstrated that in animals poisoned by plague toxin there is an inhibition of oxydation of pyruvic acid. The inhibiting action of plague toxin on oxydation can be eliminated by means of the preliminary administration of vitamin B_1 (thiamine) to white mice. The mechanism of damage of the pyruvateoxydase system during poisoning by plague still remains studied poorly. One of the means of conversion of pyruvic acid in the organism of an animal is its reverse restoration into lactic acid. This process is catalyzed by the enzyme lactatedehydrogenase (LDG). In the literature we did not find any data relative to the influence of plague microbe toxin on the activity of LDG.

In the present investigation we undertook the task to study the activity of pyruvate oxydase (in the presence of thiamine and pantothenic acid) and LDG in animals which had been poisoned with plague microbe toxin.

Methods of Investigation

The experiments were set up on white mice weighing 18—20 grams and white rats weighing 180—250 grams. The white mice were divided into three groups. For 15 days the animals of the 1st group were given pantothenic acid subcutaneously: 66 μg of calcium pantothenate in 0.2 ml of physiological solution; the second group, daily for 15 days, received 4 μg of thiamine subcutaneously; the third group of white mice were the control.

Subsequently the test and control animals (white mice) received intraperitoneally 10 LD_{50} of microbial toxin, which was obtained by the method of Baker [3] and purified by the method of Ajl [4]. In 3—4 hours after administration of the toxin, at the moment of development of acute intoxication (when 1 or 2 animals died), the animals were sacrificed and the liver rapidly removed and frozen. The activity of pyruvateoxydase was determined by oxygen consumption

in the presence of pyruvic acid with liver homogenates. Oxygen consumption was measured by the manometric method [5].

In other experiments a study was made of the influence of thiamine and calcium pantothenate on the oxidation of pyruvic acid in vitro. For this purpose healthy white mice were given 10 LD_{50} of plague toxin and in 3—4 hours the animals were sacrificed and liver homogenates were obtained. They were placed directly into vessels of a Warburg apparatus and in some tests thiamine in doses from 5 to 0.00005 μg was added, and in others - calcium pantothenate in doses of 5 to 0.025 μg.

Activity of LDG was studied on white rats, which received intraperitoneally 1 LD_{50} of plague toxin. The animals were also decapitated, the blood collected and centrifuged, and the resulting serum taken for investigation.

Extracts were prepared by means of pulverization of tissues of brain and liver in a refrigerated homogenizer with 12 volumes of an 0.15 M solution of NaCl and subsequent centrifugation. In the in vitro experiments the toxin in doses from 0.1 to 5 mg was added directly to the serum and to extracts obtained from the liver and brain.

For determination of the activity of LDG we used the method of Sevela and Tovarek [6] in the modification of Dobrinskaya and Rubina [7], and also the method of Broida and Berger [8]. The activity of LDG was expressed in micromoles of pyruvate formed in one hour under the influence of 1 ml of whole blood or 1 g of raw tissue. Healthy animals served as the control.

Results of Investigations

As seen in Fig. 1 (average data of 4—5 tests), with the addition of various doses of calcium pantothenate in vitro the consumption of oxygen by liver homogenates of white mice at the expense of pyruvic acid was diverse. Calcium pantothenate in doses of 5 to 0.1 g exerts an inhibiting effect on the oxidation of pyruvate both in healthy animals and in those poisoned with plague toxin. However, with the introduction of 0.05 g of calcium pantothenate in the tests an intensified oxygen consumption is observed for the liver homogenates of healthy animals.

It is interesting to note that a dose of 0.05 μg of calcium pantothenate also increased the oxidation of pyruvate in tests with liver homogenates from animals which were contaminated with toxin, but by a very insignificant value. In healthy white mice oxygen

consumption reached 8.9 micromoles, and in contaminated mice - 3.9 micromoles. Therefore, in all subsequent tests we used a dose of calcium pantothenate equal to 0.05 μg.

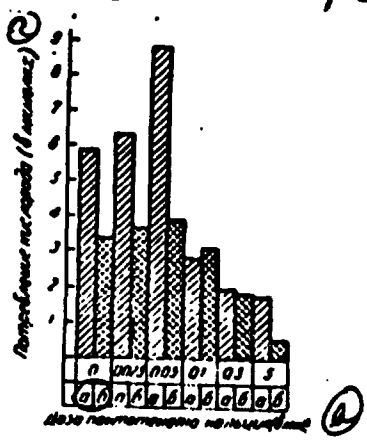

Fig. 1. Influence of dose of calcium pantothenate on oxygen consumption by liver homogenates of healthy (a) and plague toxin contaminated (b) white mice at the expense of pyruvic acid.
Key: (a) Oxygen consumption (in micromoles); (b) Dose of calcium pantothenate (in μg): a - a, b - б.

Table 1

Influence of calcium pantothenate on oxygen consumption by liver homogenates of healthy and plague toxin contaminated white mice

(a) Животные	Потребление O_2 (в микромолях) (b)		
	в пробах с пируватом (A) (c)	в пробах без пирувата (B) (d)	за счет пирувата (A—B) (e)
(f) Здоровые (36)	16,7±0,7	10,8±0,7	5,9
(g) Отравленные (15) . . .	11,6±0,4	8,4±0,8	3,2
(h) Здоровые, получавшие пантотенат кальция (14)	21,9±0,4	12,1±0,7	9,8
(i) Отравленные, получавшие пантотенат кальция (6)	8,3±0,5	5,6±0,8	2,7
(j) Здоровые, пантотенат кальция добавлен in vitro (14)	22,7±1,1	12,9±1,4	9,8
(k) Отравленные, пантотенат кальция добавлен in vitro (7)	11,2±0,9	9,0±1,0	2,2

Key: (a) Animals; (b) O_2 consumption (in micromoles); (c) in tests with pyruvate (A); (d) in tests without pyruvate (B); (e) due to pyruvate (A—B); (f) Healthy (36); (g) Contaminated (15); (h) Healthy, received calcium pantothenate (14); (i) Contaminated, received calcium pantothenate (6); (j) Healthy, calcium pantothenate added in vitro (14); (k) Contaminated, calcium pantothenate added in vitro (7).
Note: Here and in Table 2 the figures in parenthesis are the number of tests.

Table 1 shows that after the introduction of calcium pantothenate in doses of 0.05 μg into the tests, and also after the daily administration of it to healthy mice in doses equal to 66 μg, in the course of 15 days the absorption of oxygen by liver homogenates in the test probes was considerably greater than in the control. The difference in oxygen consumption due to pyruvate in the tests with pantothenate and without it is very significant ($P<0.001$). Beside this, in the presence of calcium pantothenate both in tests in vitro and in vivo a tendency is observed for an increase of endogenic respiration.

As can be seen from Table 1, calcium pantothenate does not increase oxidation of pyruvic acid in tests with liver homogenates of contaminated animals. Moreover the overall consumption of oxygen by liver homogenates from animals which had received in vivo calcium pantothenate, after the administration of toxin of the plague microbe, became considerably lower than in the control tests.

Inhibition of oxygen consumption is also observed due to endogenic respiration (difference is statistically reliable, $P<0.01$).

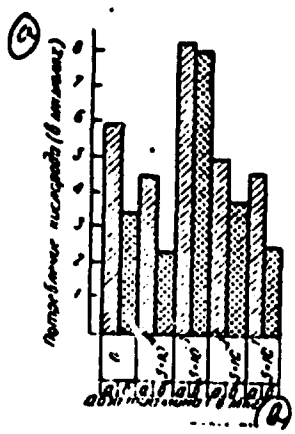

Fig. 2. Influence of dose of vitamin B_1 on oxygen consumption by liver homogenates of healthy (left column) and plague toxin contaminated (right column) white mice at the expense of pyruvic acid. Key: (a) Oxygen consumption (in micromoles); (b) Dose of thiamine (in μg).

In another series of tests a study was made of the influence of various doses of thiamine on oxidation of pyruvic acid in healthy and plague toxin contaminated white mice.

In Fig. 2 (average data of 4--5 tests) it can be seen that thiamine, administered in tests in vitro in various amounts, just as calcium pantothenate, has an effect on the oxidation of pyruvate which, depending on dose, is different. Here a specific dependence exists between oxygen consumption (due to oxidation of pyruvic acid) and the concentration of thiamine. The addition of thiamine in the tests

both in large and in small concentrations inhibits the absorption of oxygen by liver homogenates of healthy animals and those contaminated with toxin of the plague microbe.

Table 2

Influence of vitamin B_1 on oxygen consumption by liver homogenates of healthy and plague toxin contaminated white mice

(a) Животные	(b) Потребление O_2 (в микромолях)		
	в пробах с пируватом (A)	в пробах без пирувата (B)	за счет пирувата (A—B)
(f) Здоровые (36)	16,7±0,7	10 8±0,7	5,9
(g) Отравленные (15) . . .	11,6±0,4	8,4±0,8	3,2
(h) Здоровые, получавшие витамин B_1 (5) . . .	18,9±0,5	11,7±0,7	7,2
(i) Отравленные, получавшие витамин B_1 (9)	16,6±0,4	9,6±0,5	7,0
(j) Здоровые, витамин B_1 добавлен in vitro (15)	21,2±0,9	13,2±0,7	8,0
(k) Отравленные, витамин B_1 добавлен in vitro (3)	19,8±0,7	12,9±0,5	6,9

Key: (a) Animals; (b) Consumption of O_2 (in micromoles); (c) in tests with pyruvate (A); (d) in tests without pyruvate (B); (e) due to pyruvate (A—B); (f) Healthy (36); (g) Contaminated (15); (h) Healthy, received vitamin B_1 (5); (i) Contaminated, received vitamin B_1 (9); (j) Healthy, vitamin B_1 added in vitro (15); (k) Contaminated, vitamin B_1 added in vitro (3).

As our experiments showed, with a concentration of thiamine equal to 0.005 μg the absorption of oxygen by liver homogenates reached a maximum. Oxygen consumption by liver homogenates of healthy animals comprised 8.2 micromoles, and of animals contaminated with plague toxin - 7.8 micromoles. Particularly noteworthy is the fact that thiamine in doses equal to 0.005 μg (Table 2) stimulates the oxidation of pyruvate not only in tests with liver homogenates of healthy animals, but also in tests with liver homogenates of white mice which were contaminated with plague toxin. Here the consumption of oxygen by liver homogenates from animals which were contaminated with plague toxin turned out to be somewhat higher than in the control. There is also a tendency for an increase of endogenic respiration.

Table 2 shows that the preliminary administration of thiamine to white mice also leads to an increase in the consumption of oxygen by liver homogenates of the animals. Here the consumption of oxygen by liver homogenates of healthy animals, which had been given thiamine for a prolonged period of time, was increased by 1.3 micromoles. The amount of oxygen which was absorbed by liver homogenates of contaminated animals which had received thiamine remained the same as in the control.

These data completely agree with the results of investigations published by us earlier [2]. However, they are more convincing, since the tests were conducted on white mice which had been contaminated with a relatively large dose of plague toxin (10 LD_{50}) and the animals were sacrificed at the moment of development of acute intoxication.

Figure 3 shows the results of a study of the direct action of plague microbe toxin on LDG of tissues of white rats in vitro.

Plague microbe toxin in doses of 0.1 to 5 mg did not change the activity of LDG in the investigated tissues. Fluctuations noted in the activity of LDG are not statistically reliable.

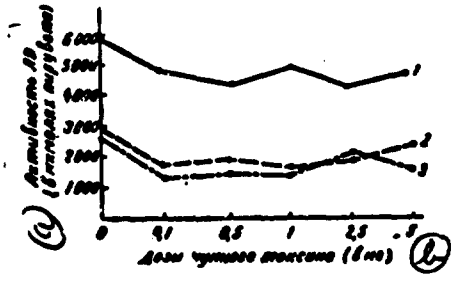

Fig. 3. Activity of lactatedehydrogenase of tissues during administration in tests of various doses of plague microbe toxin.
1 - liver extract; 2 - brain extract; 3 - blood serum.
Key: (a) Activity of LDG (in micromoles of pyruvate); (b) Dose of plague microbe (in mg).

Table 3

Activity of LDG of rat tissues under normal conditions and during plague intoxication (in micromoles of pyruvate for 1 hour for 1 g of tissue

(a) Объект исследования	(b) Норма: (d) число опытов	(b) Норма: (e) активность ЛДГ	(c) Интоксикация: (d) число опытов	(c) Интоксикация: (e) активность ЛДГ	(f) Достоверность (T)
(g) Печень	27	5 980±312	15	6 812±333	2,1
(h) Мозг	22	2 964±190	12	1 950±166	3,2
(i) Кровь+НАД	13	2 756±117	10	3 406±130	3,0
(j) Кровь+НАД-H_2	6	15 834±457	11	10 452±530	4,6

Key: (a) Object of investigation; (b) Normal; (c) Intoxication; (d) number of tests; (e) activity of LDG; (f) Reliability (T); (g) Liver; (h) Brain; (i) Blood + NAD; (j) Blood + NAD-H_2 *.

* Transliterated from Cyrillic - reference in text to coenzyme of LDG.

Extracts of brain and liver, and also blood, dehydrogenated lactate at the same rate both in the presence and in the absence of plague microbe toxin.

The results of the experiments on the study of the activity of LDG in the tissues of rats during plague intoxication are presented in Table 3. It turned out that the activity of the enzyme in the liver, brain, and blood serum in white rats which were contaminated with plague microbe toxin is changed non-equivalently. In the brain the activity of LDG is noticeably reduced, in the serum it is increased, and in the liver it remains without change.

The activity of LDG of blood serum was determined by the accumulation of pyruvate in the test in the presence of NAD [7] or by the diminuation of pyruvic acid in the presence of NAD-N_2 [8]. The rate of the reaction was changed similarly in both cases.

Discussion of Results

The resulting data on the influence of thiamine and pantothonic acid on the oxidation of pyruvate permit the assumption that the toxin of plague microbe prevents the formation of thiamine pyrophosphate, thus blocking the process of oxidizing decarboxylation of pyruvic acid. As can be seen from the data obtained, thiamine, but not calcium pantothenate, restores the process of oxidizing decarboxylation of pyruvate which was disrupted by the plague microbe toxin. It is still difficult to say if the action of the plague toxin on the pyruvate-oxydase system is specific.

It can be thought that the toxin of the plague microbe inhibits the oxidation of pyruvate by means of dephosphorylation of cocarboxylase, as a result of which thiamine pyrophosphate is not formed. In confirmation of this assumption it is possible to refer to the results obtained in the investigations by Greig and Govier [9]. Those authors studied the activity of cocarboxylase in the muscles, liver, and kidneys of dogs, in which a state of hemorrhagic shock was caused artificially, and also oxygen deficiency of the type of hypoxic hypoxia. They established that during hemorrhagic shock and hypoxia a dephosphorylation of cocarboxylase takes place. By means of the administration of vitamin B_1 to animals the authors were able to increase the resistance of cocarboxylase to decomposition. On the other hand, in the opinion of a number of authors a leading role in the pathogenesis of plague and intoxication belongs to shock phenomena [10—14].

During plague intoxication, as noted by Kratinov and Khar'kova [15], along with a change in the content of sugar in the blood of white rats and guinea pigs there is an increase in the concentration of lactic acid.

In tests on white mice Dzhaparidze and Sidorova [16] showed that during plague and plague intoxication changes are also observed in the content of lactic acid in various tissues. According to their data in the brain of animals the amount of lactic acid was reduced by more than two times following the administration of toxin of the

plague microbe. Oppression of LDG activity in the brain and intensification of it in blood serum in our tests can also be explained, apparently, by the change in the concentration of substrates of this enzyme. Considering the changes detected by us in the activity of LDG and the data from the literature concerning disruptions in the metabolism of lactic acid in animals during plague intoxication, we proposed that in the tissues of sick animals quantitative disruptions are possible in the content of the coenzyme of lactatedehydrogenase - NAD.

By means of the simultaneous administration of large doses of NAD with the toxin we attempted to prevent the diminuation of the NAD in the organs and tissues and to determine how this is reflected on the sensitivity of the animals to the toxin of the plague microbe. Preliminary tests showed that a single administration of 10 mg of NAD (in 0.2 ml of physiological solution) to white mice simultaneously with toxin of the plague microbe (1 LD_{50}) protected all the animals from death in the first 2 days, while in the control half of the animals died. In the next 10 days out of the 20 animals which had received NAD only 5 died, while in the control 16 out of 20 animals died. Consequently, under the influence of NAD the resistance of white mice to the toxin of the plague microbe is increased.

It is necessary to note that an increase of resistance in white mice to the toxin of the plague microbe is also observed following the administration of the coenzyme of the pyruvateoxydase system - thiamine [17]. Thus the normalization of metabolism of pyruvate and lactate following the administration of thiamine and NAD, and also the increase in the resistance of the animals to the toxin of the plague microbe, give a foundation to assume that damage to the pyruvateoxydase and lactatedehydrogenase enzyme systems plays a definite role in the pathogenesis of plague intoxication. It is known that in the treatment of plague vitamin B_1 is included in the list of vitamins which are recommended for administration to patients. The data obtained by us confirm the foundation for the use of vitamin B_1 in the treatment of plague infection. Zakharova, Dzhaparidze, and Ryshkina [18] also note that combined treatment of experimental plague with antibiotics with vitamin B_1 is more effective than without it.

Conclusions

1. Calcium pantothenate, administered subcutaneously and added in tests in vitro, intensifies oxygen consumption by liver homogenates of healthy white mice and reduces consumption of it by liver homogenates of white mice which are contaminated with plague toxin.

2. Thiamine, administered subcutaneously and added in samples, stimulates oxidation of pyruvate by liver homogenates of healthy animals and restores to normal the oxygen consumption of liver homogenates of animals which are contaminated with the toxin of the plague microbe.

3. Plague microbe toxin, added directly to extracts of liver, brain, and serum, does not exert an influence on lactatedehydrogenase activity.

4. Under the influence of plague microbe toxin, administered to animals intraperitoneally, lactatedehydrogenase activity is intensified in blood serum, is suppressed in the brain, and remains without change in the liver.

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